

**In the Claims:**

1. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, a multipli-charged inorganic anion, a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0, said product being present in said solution in a sufficient amount to provide a therapeutically effective amount of said product when the solution is administered to a patient, said liquid composition being stable at room temperature for at least about six months and not containing urea.

2. (Original) The composition of claim 1 wherein said solution is an isotonic solution.

3. (Currently amended) The composition of claim 1 wherein the anion is an anion of a ~~multiply-charged~~ multipli-charged strong inorganic acid.

4. (Original) The composition of claim 3 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

5. (Original) The composition of claim 4 wherein the anion is a sulfate anion.

6. (Original) The composition of claim 1 wherein the pH is 5.8 to 6.7
7. (Original) The composition of claim 6 wherein the pH is 6.0 to 6.5
8. (Original) The composition of claim 7 wherein the pH is about 6.2.
9. (Original) The composition of claim 1 wherein the buffer is selected from the group consisting of a phosphate or arginine/H<sub>2</sub>SO<sub>4</sub>/Na<sub>2</sub>SO<sub>4</sub> buffers.
10. (Original) The composition of claim 9 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.
11. (Previously presented) The composition of claim 1 wherein the product is a human erythropoietin.
12. (Canceled)
13. (Currently amended) The composition of claim 11[12] wherein the erythropoietin has the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.
14. (Previously presented) The composition of claim 13 wherein the amino acid sequence of the erythropoietin is modified by the addition of from 1 to 6 glycosylation sites.

15. (Previously presented) The composition of claim 14 wherein the sequence modification is

Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>.

16. (Previously presented) The composition of claim 13, wherein the erythropoietin has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

17. (Original) The composition of claim 16, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin sequence with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

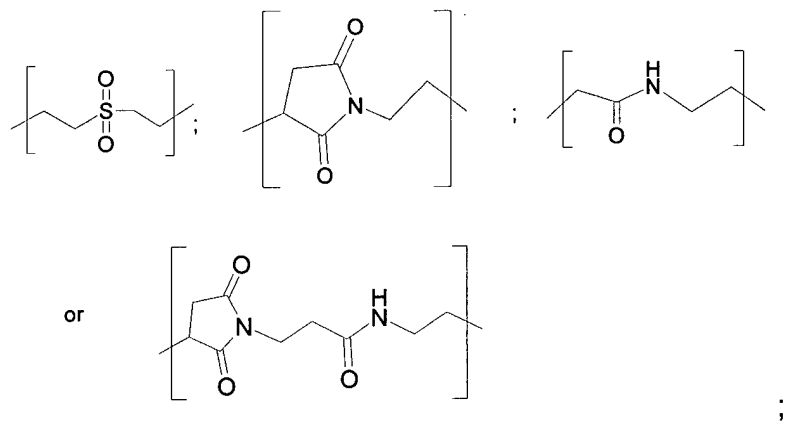
18. (Cancelled)

19. (Original) The composition of claim 1, wherein the glycoprotein product is a pegylated erythropoietin.

Claims 20-22 (Cancelled)

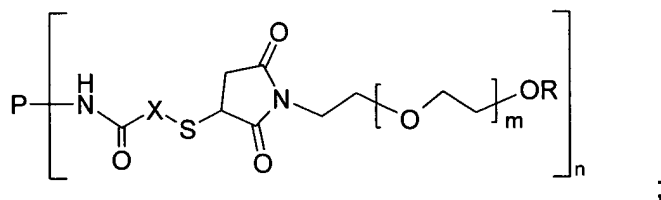
23. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising a pegylated erythropoietin glycoprotein conjugate, said glycoprotein having at least one free amino group and having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being

covalently linked to the glycoprotein *via* a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups; X is -(CH<sub>2</sub>)<sub>k</sub>- or -CH<sub>2</sub>(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>k</sub>-; k is from 1 to 10; Y is selected from



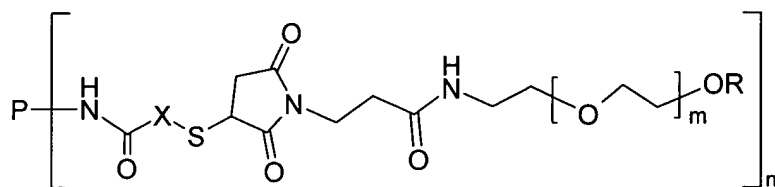
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons; and wherein the molecular weight of the pegylated erythropoietin glycoprotein is from about 51 kilodaltons to about 175 kilodaltons; said liquid composition being stable at room temperature.

24. (Previously presented) The composition of claim 23 wherein said conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ ; and P is the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is from 1-10.

25. (Previously presented) The composition of claim 23 wherein the conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower alkyl; X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ ; and P is the erythropoietin glycoprotein without the amino group or groups which form an amide linkage; and k is from 1-10.

26. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from 10  $\mu$ g to 10,000  $\mu$ g per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, from 10 to 200 mmol per liter of said solution of a multipli-charged inorganic anion and from 10 to 50 mmol per liter of said solution of a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount to provide the solution with a pH of from 5.5 to about 7.0; said liquid pharmaceutical composition being stable at room temperature for at least about six months and not containing urea.

27. (Original) The composition of claim 26 wherein said solution is an isotonic solution.

28. (Previously presented) The composition of claim 26 wherein the anion is an anion of a multipli-charged strong inorganic acid.

29. (Original) The composition of claim 28 wherein the anion is selected from the group consisting of sulfate, citrate or phosphate.

30. (Original) The composition of claim 29 wherein the anion is a sulfate anion.

31. (Original) The composition of claim 30 wherein the pH is 5.8 to 6.7

32. (Original) The composition of claim 30 wherein the pH is 6.0 to 6.5

33. (Original) The composition of claim 31 wherein the pH is about 6.2.

34. (Previously presented) The composition of claim 26 wherein the buffer is selected from the group consisting of phosphate or arginine/H<sub>2</sub>SO<sub>4</sub>/Na<sub>2</sub>SO<sub>4</sub> buffers.

35. (Original) The composition of claim 34 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.

36. (Original) The composition of claim 26 wherein the product is a human erythropoietin.

37. (Cancelled)

38. (Currently amended) The composition of claim 36 [37] wherein the erythropoietin has the amino acid sequence SEQ ID NO:1 or SEQ ID NO:2.

39. (Previously presented) The composition of claim 26 wherein said erythropoietin glycoprotein product has the sequence SEQ ID NO: 1 or SEQ ID NO: 2 that is modified by the addition of from 1 to 6 glycosylation sites.

40. (Previously presented) The composition of claim 39 wherein the sequence of modification is

Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>.

41. (Previously presented) The composition of claim 26 wherein said erythropoietin glycoprotein product has the sequence SEQ ID NO: 1 or SEQ ID NO: 2 that is modified by a rearrangement of at least one glycosylation site.

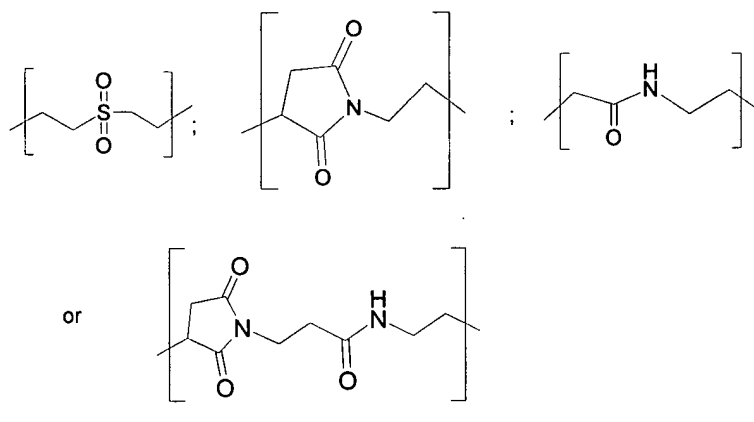
42. (Original) The composition of claim 41, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

43. (Cancelled)

44. (Previously presented) The composition of claim 26, wherein said glycoprotein product is a pegylated erythropoietin glycoprotein product.

Claims 45-47 (Cancelled)

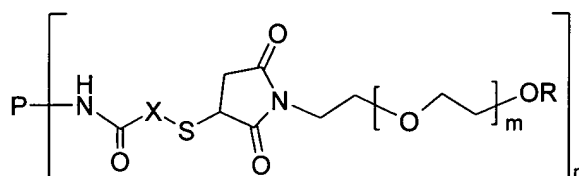
48. (Previously presented) The composition of claim 44, wherein the pegylated erythropoietin glycoprotein product is a conjugate of an erythropoietin glycoprotein having at least one free amino group, said glycoprotein having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein *via* a linker of the formula  $-C(O)-X-S-Y-$  with the  $C(O)$  of the linker forming an amide bond with one of said amino groups, X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ ; and k is from 1 to 10; Y is selected from





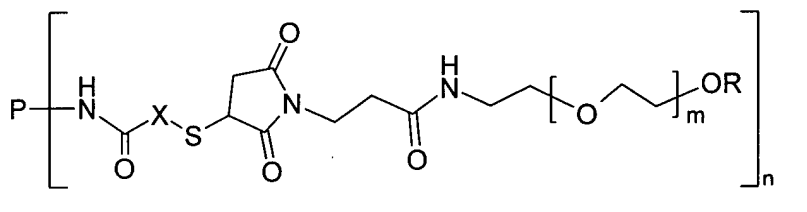
the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons; and the molecular weight of the conjugate being from about 51 kilodaltons to about 175 kilodaltons.

49. (Previously presented) The composition of claim 48 wherein said conjugate has the formula:



wherein  $n$  is an integer from 1 to 3;  $m$  is an integer from 450 to 900;  $R$  is lower alkyl;  $X$  is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , and  $P$  is the erythropoietin glycoprotein minus the amino group or groups which form an amide linkage with  $X$  and  $k$  is from 1 to 10.

50. (Previously presented) The composition of claim 49 wherein said conjugate has the formula:



$n$  is an integer from 1 to 3;  $m$  is an integer from 450 to 900;  $R$  is lower alkyl;  $X$  is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , and  $P$  is the erythropoietin glycoprotein minus the amino group or groups which form an amide linkage; and  $k$  is from 1 to 10.

51. (Previously presented) The composition of claim 26 wherein said solution contains 10 µg to 10000 µg erythropoietin protein per ml of solution, from 10 to 200 mmol/liter of solution of a sulfate as the multiply charged inorganic anion, and 10 to 50 mmol/liter of solution of a phosphate as the pharmaceutically acceptable buffer, said solution having a pH of from about 6.0 to about 6.5.

52. (Previously presented) The composition of claim 51 further comprising up to 20 mM of methionine, and 1 - 5 % of a polyol (w/v).

53. (Previously presented) The composition of claim 52 comprising 10 µg to 10000 µg erythropoietin protein per ml of solution, 40 mmol/liter of solution of the sulfate, 10 mmol/liter of said solution of the phosphate, 10 mM methionine, said composition having a pH of about 6.2, and wherein the polyol is mannitol which is present in the solution at 3% (w/v).

54. (Previously presented) The composition of claim 26 wherein the solution contains 10 µg to 10000 µg erythropoietin protein per ml of solution, the buffer is phosphate which is present at 10 to 50 mmol/liter of solution, said solution further comprising NaCl which is present at 10 to 100 mmol/liter of solution and having a pH of from about 6.0 to about 7.0.

55. (Previously presented) The composition of claim 54 wherein the NaCl is present at 100 mmol/liter of solution, the phosphate is present at 10 mmol/l, said solution further comprising 10 mM methionine and having a pH of about 7.0.

Claims 56 - 58 (Cancelled)

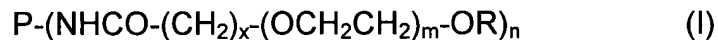
59. (Previously presented) The composition of claim 26 wherein the amount of erythropoietin is 50, 100, 400, 800 or 2,500 µg/ml of solution.

60. (Previously presented) The composition of claim 59 comprising 10 mM sodium phosphate and 40 mM sodium sulfate and further comprising 3% mannitol, 10 mM methionine and 0.01% pluronic F68, and having a pH 6.2.

61. (Previously presented) The composition of claim 59 comprising 40 mM arginine and 30 mM sodium sulfate and further comprising 3% mannitol, 10 mM methionine, 0.01% pluronic F68, and a pH 6.2.

Claims 62-66 (Cancelled)

67. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising a therapeutically effective amount of a pegylated erythropoietin glycoprotein product of formula



wherein

P is an erythropoietin glycoprotein having the sequence SEQ ID NO: 1, SEQ ID NO: 2, or either of these sequences modified by the addition of from 1 to 6 glycosylation sites or by rearrangement of at least one glycosylation site, minus the n amino group of said glycoprotein,

R is lower alkyl,

x is 2 or 3,

m is from about 450 to about 900;

n is from 1 to 3; and

wherein the values of n and m are such that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons; and

a multiply charged inorganic anion and a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount such that the pH of the solution is from about 5.5 to about 7.0.

68. (Previously presented) The composition of claim 67 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

69. (Previously presented) The liquid pharmaceutical composition of claim 67 wherein the pegylated erythropoietin glycoprotein product is present in an amount of from about 10  $\mu\text{g}$  to about 10,000  $\mu\text{g}$  per ml of said liquid composition, the multiply charged inorganic anion is present in an amount of from 10 to 200 mmol per liter of said liquid composition, and the pharmaceutically acceptable buffer is present in an amount of from about 10 to about 50 mmol per liter of said liquid composition, said anion and said buffer being present in said liquid composition in an amount to provide a pH of from about 5.5 to about 7.0.

70. (Previously presented) The composition of claim 69 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

71. (Currently amended) The liquid pharmaceutical composition of claim 68 wherein the pegylated erythropoietin glycoprotein is present in an amount of about

100.0  $\mu\text{g/mL}$  of solution, the multiply charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about  $6.2^{[\pm]} \pm 0.2$ .

72. (Previously presented) The liquid pharmaceutical composition of claim 71 further comprising methionine in an amount of about 1.49 mg/mL, mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

73. (Currently amended) The liquid pharmaceutical composition of claim 68 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 400  $\mu\text{g/mL}$  of solution, the multiply charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about  $6.2^{[\pm]} \pm 0.2$ .

74. (Previously presented) The liquid pharmaceutical composition of claim 73 further comprising methionine in an amount of about 1.49 mg/mL, mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

75. (Currently amended) The liquid pharmaceutical composition of claim 68 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 800.0  $\mu\text{g/mL}$  of solution, the multiply charged inorganic anion is sodium sulphate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about  $6.2^{[\pm]} \pm 0.2$ .

76. (Currently amended) The liquid pharmaceutical composition of claim 75 further comprising methionine in an amount of about 1.49 mg/mL, mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

77. (Previously presented) The composition of claim 26 wherein the erythropoietin is present at about 25 to about 2,500 µg/ml, the buffer is sodium or potassium phosphate which is present in an amount of about 10 mM, said composition further comprising NaCl which is present in an amount of about 100 mM and having a pH of about 7.0.

Claims 78 – 82 (Canceled)

83. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from 10 µg to 10,000 µg per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, from 10 to 100 mmol/liter of solution of sodium sulfate, and from 10 to 50 mmol/liter of said solution of arginine, said solution having a pH of from about 6 to about 6.5 and being stable at room temperature.

84. (Previously presented) The composition of claim 83 wherein said solution comprises 40 mmol/liter of solution of arginine, 30 mmol/liter of solution of sodium sulfate, said solution further comprising 3% mannitol, 10 mM methionine, and 0.01% pluronic F68, and having a pH of about 6.2.

85. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from about 25  $\mu\text{g}$  to about 2,500  $\mu\text{g}$  per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, about 120 mM of sodium sulfate, and about 10 mM of sodium phosphate, said solution having a pH of about 6.2 and being stable at room temperature.

86. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from about 25  $\mu\text{g}$  to about 2,500  $\mu\text{g}$  per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, about 40 mM of sodium sulfate, and about 10 mM sodium phosphate, said solution having a pH of about 6.2 and being stable at room temperature.

87. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from about 25  $\mu\text{g}$  to about 2,500  $\mu\text{g}$  per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, about 40 mM of sodium sulfate, about 10 mM sodium phosphate, 3% mannitol and 10 mM methionine, and having a pH of about 6.2 and being stable at room temperature.

88. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from about 25  $\mu\text{g}$  to about 2,500  $\mu\text{g}$  per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells,

Serial No. 09/853,731  
Filed: May 11, 2001

about 30 mM of sodium sulfate, about 40 mM arginine, and 3% mannitol, said solution having a pH of about 6.2 and being stable at room temperature.

89. (Previously presented) A liquid pharmaceutical composition in the form of an aqueous solution comprising from about 25  $\mu\text{g}$  to about 2,500  $\mu\text{g}$  per ml of said solution of an erythropoietin glycoprotein product having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, about 30 mM of sodium sulfate, about 40 mM arginine, and about 3% mannitol, said solution having a pH of about 6.2 and being stable at room temperature.